NANO-ENCAPSULATION OF SELECTED DRY FRUITS WITH GREEN NANOPARTICLES FOR ENHANCING THEIR SHELF LIFE.

Research Protocol

For Registration in Ph. D. Program

Submitted by

Reshma Kapoor

Under the Guidance Of

Prof. (Dr.) Widhi Dubey

Department of Botany

JECRC UNIVERSITY, JAIPUR

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Dated : ……

CERTIFICATE

This is to certify that this research protocol entitled **"NANO-ENCAPSULATION OF SELECTED DRY FRUITS WITH GREEN NANOPARTICLES FOR ENHANCING THEIR SHELF LIFE."** by RESHMA KAPOOR submitted in fulfilment of the requirement for registration in Ph. D Program in Department Of Botany under School Of Science, JECRC University, Jaipur, during the academic year 2023-2024, is a bonafide record work carried out under my guidance and supervision.

Prof. (Dr.) Widhi Dubey

Department of Botany

JECRC University, Jaipur

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1. Introduction

Dry fruits and nuts are being consumed worldwide for many years due to their high nutritional content, enjoyable flavor, and significance in the pharmaceutical and cosmetic industries (Benedict et al., 2016). Nevertheless, there is much documentation on both the allergic reactions caused by these nuts and the potential health hazards associated with long-term and excessive consumption of mycotoxins found in dried fruits and nuts (Ismail et al., 2021). Allergy to dry fruits or nuts is a condition characterized by hypersensitivity to certain organic components found in them. On the other hand, the menace of mycotoxicosis is associated with the overall health of the dry fruit or nut. Mycotoxins, which are harmful substances, are produced and secreted by different types of fungi that can infect dried fruits and nuts at every stage of production, starting from the farm and ending with consumption (Wang et al., 2018).

Nuts and dried fruits possess significant nutritional value when consumed either roasted or fresh. They are particularly abundant in protein, fat, carbohydrates, amino acids, and vitamins. A wide range of dried fruits and nuts are extensively employed in many sectors such as confectionery, cosmetics, resins, and are also consumed directly. Due to their high protein and fat content, as well as low water content, various nuts such as "hazelnut (*Corylus avellana*), cashew (*Anacardium occidentale* L), almond (*Prunus dulcis*), pistachio (*Pistacia vera* L), and peanut (*Arachis hypogaea*)" are susceptible to attack by pre and/or post-harvest molds (Bayman et al., 2002; Magnoli et al., 2004; Jorgensen, 2005). If nuts are marketed and stored improperly, mold may proliferate fast. Contamination of nuts with fungus can occur at three stages. Firstly, while these fruits are still on the tree, they are susceptible to being attacked by spores of various fungus species that are present in the air. Secondly, contamination can occur during the de-hulling and washing process after the nuts have been harvested. Typically, the last step often happens during storage, particularly when nuts are inadequately dried or kept in harsh temperature and humidity conditions. Currently, there are hundreds of fungal species that are categorized as natural pollutants of agricultural and food goods. These species include toxigenic and pathogenic fungi (Pitt et al., 1993).

Mycotoxins are poisonous byproducts synthesized by different species of fungi, which have the potential to pollute crops, so impacting both public health and the agricultural economy. Mycotoxins exert a substantial and detrimental impact on health, with their effects being dependent on the specific kind and quantity of toxins present in contaminated materials. Additionally, other environmental and essential elements might influence the response of individuals exposed to these toxins (Hocking et al., 2007).
The occurrence of mycotoxins is escalating when many types of toxins are present in the same agricultural or food item. Many fungal species possess the capacity to create many types of toxins, whereas various fungal species can also release identical mycotoxins. Multiple investigations have demonstrated the existence of many mycotoxins in food specimens, which carries significant consequences for both the safety of food and the well-being of individuals (Smith and Groopman, 2019). Over 300 mycotoxins have been discovered, with approximately twelve receiving the greatest scrutiny because of their significant effects on human health and their occurrence in food. Aflatoxins (AFs) are considered to be the most carcinogenic naturally occurring compounds compared to other mycotoxins. "*Aspergillus flavus, A. parasiticus, A. nomius,* and other species" are molds that produce toxins and have the ability to thrive in many types of nuts such as almonds, cashews, hazelnuts, and peanuts (Ismail et al., 2018).

Mycotoxins encompass a diverse array of organic compounds, typically exhibiting low molecular weight. They possess remarkable bioactivity even at minuscule concentrations, measured in parts per billion (ppb) or trillion (ppt), equivalent to micrograms per liter (μg/L) and nanograms per liter (ng/L) respectively. These compounds exhibit exceptional stability, displaying resistance to degradation, and are synthesized through the secondary metabolic pathways of various filamentous fungi, commonly referred to as molds. When microbes infect and proliferate on food and feed, they secrete toxic molecules that, upon ingestion by humans or animals, can lead to a range of acute (such as rash, vomiting, diarrhea,

headache, etc.) or chronic (including nephropathies, immunosuppression, carcinogenic effects, etc.) adverse effects (Freire and da Rocha, 2016).

The potential health hazards can stem from their carcinogenic properties, as reported by International Agency for Research on Cancer (IARC) based on study of some mycotoxins in humans (Ostry et al., 2017). To safeguard consumers from excessive exposure to highly hazardous mycotoxins, numerous countries and federations of states have implemented regulations that establish maximum allowable levels in a diverse range of food products intended for human and animal consumption. For instance, the European Union has enacted Commission Regulation (EC) No 1881/2006, as published in the Official Journal of the European Union, L. 364/5, on December 20, 2006, along with Commission Regulation (EC) No 401/2006, as published in the same journal, L70/12, on March 9, 2006, and subsequent revisions (Mirabile et al., 2021).

Mycotoxins have the potential to provide various hazards to the health of both humans and animals. The concerns pertaining to health have frequently been highlighted; yet the precise processes via which these toxins induce such detrimental effects remain inadequately elucidated. Regardless, the amount and length of time an individual is exposed to mycotoxins, the interaction between different fungal metabolites, the genetic predisposition and physical characteristics of the individual, and other factors, can significantly influence the expression of their hazardous effects (Abbas et al., 2019).

According to Bayman et al. (2002), the predominant fungal species found in nuts were "*Aspergillus, Rhizopus,* and *Penicillium. A. niger, A. flavus, A. parasiticus,* and *A. nomius"* were identified in surveys conducted throughout several regions including the United States, Europe, Australia, and South America (Mangoli et al., 2004; Jorgensen, 2005; Rodrigues et al., 2012). The problem lies in the presence of several species of aspergilli and penicillia, such as *A. niger, A. flavus*, and *Penicillium crustosum*, as it has been demonstrated that certain members of these species have the ability to produce mycotoxins. The *Aspergillus* section *Flavi*, which includes *A.*

flavus, A. parasiticus, and *A. nomius*, is recognized for its ability to synthesize aflatoxins (AFs), which are toxic and carcinogenic compounds found in various food and agricultural products, particularly tree nuts. On the other hand, the black aspergilli (*Aspergillus* section *nigri*) produce a total of 145 secondary metabolites, some of which have been identified as harmful to both humans and animals. To be more precise, *Aspergillus niger* has the capability to generate ochratoxin A (OTA) and fumonisin B2 (FB2), whereas the majority of strains of "*Aspergillus carbonarius* are known to primarily produce OTA. *Aspergillus* section *nigri* often produces substantial quantities of malformins, naphtho-γ-pyrones, and bicumarins (kotanins), which are potentially hazardous metabolites". Tree nuts have been shown to contain naturally occurring mycotoxins, including AFs, OTA, and FB2, as described in previous studies. A limited number of pick-out almond samples were found to have trace amounts (less than 1.5 ng g-1) of FB2.The presence of 8 aflatoxins (ranging from 15 to 25 ng g-1) and zearalenone (125 ng g-1) was detected in walnuts. Almonds have been found to have elevated amounts of AFs, reaching up to 95 ng g-1. Additionally, the presence of stergmatocystin (ST) has been identified in in-shell pecans (Tournas et al., 2015).

Following the harvest, fruits may have plant pathogenic species acquired from the field, with the potential for survival during the subsequent drying process. In previous studies, researchers have identified the presence of potentially toxigenic storage fungus in a range of dried fruits. Tournas (2004, unpublished data) conducted a study in which "*A. niger, A. ochraceus* and *A. carbonarius*, were identified from raisins. Similarly, Hocking et al., (2007) documented the occurrence of *Aspergillus* section *nigri* and *A. ochraceus"* in grapes and other related products, including raisins. The literature has also documented the occurrence of OTA in dried fruits, including currants, raisins, and sultanas.

Preventive measures available

At now, a variety of synthetic preservatives are being employed to mitigate the risk of contamination in food products. The literature highlights the adverse effects of synthetic preservatives, which are frequently employed to

mitigate the growth of molds and the occurrence of aflatoxin contamination. Many findings have prompted significant apprehension over the ongoing usage of these preservatives in the context of food preservation.

In addition, it has been documented that synthetic preservatives can increase the production of reactive oxygen species (ROS), thereby impacting the functionality of biomolecules such as nucleic acids, proteins, and lipids (Halliwell et al., 2000). The primary environmental factors that must be regulated to mitigate aflatoxin accumulation during storage are moisture, temperature, and relative humidity (Neme and Mohhammed, 2017). Hermetic storage is the widely used method for this purpose and it is capable of controlling on the levels of AFB1 and AFT, revealing a substantial reduction ($p < 0.05$) compared to samples stored in open air and under cold storage conditions. The hermetic storage system employs a sealed and waterproof construction, which results in the creation of an internal modified environment characterized by elevated levels of carbon dioxide and reduced levels of oxygen. This atmospheric composition is primarily attributed to the respiration process of the biotic constituents present inside the stored food product. These circumstances are not conducive to the proliferation and synthesis of aflatoxins by obligate aerobic aflatoxigenic fungi. The efficacy of hermetic storage in reducing or depleting oxygen levels within peanut storage containers was established (Navarro et al., 2012).

According to some studies, it is suggested that the optimal method for preventing the excessive buildup of aflatoxins is through the cold storage. Following this, hermetic storage is considered to be the next most successful approach. Conversely, open air storage is found to be the least effective method, as it promotes the growth of aflatoxigenic mold and subsequent synthesis of aflatoxins. Nevertheless, hermetic storage is considered the most advisable option due to its technical and economic viability in comparison to cold storage. Cold storage, although unusual for preserving food goods like the aforementioned, necessitates expensive refrigeration equipment and ongoing maintenance (Naeem et al., 2022). Therefore, food manufacturers are actively seeking safer alternatives to synthetic preservatives in order to increase the shelf life of stored food

commodities and protect them from fungal and mycotoxin contamination (Dwivedy et al., 2017). A variety of essential oils, including "*Anethum graveolens, Adenocalymma alliaceum, Caesulia axillaris, Citrus medica, Citrus maxima, Citrus sinensis, Chenopodium ambrosioides, Cymbopogon flexuosus, Foeniculum vulgare, Melaleuca leucadendron, Ocimum canum, Cymbopogon citratus, Lantana indica, Lippia alba, Mentha arvensis, Mentha spicata, Ocimum sanctum, Trachyspermum ammi, and Zanthoxylum alatum",* have been documented as having antifungal properties against a wide range of fungi associated with food contamination. The primary factor contributing to the antibacterial activity of essential oils (EOs) is often attributed to the principal compounds present or the combined action of all major and minor compounds (Dwivedy et al., 2016). In the recent years, the utilization of nano-encapsulation techniques for essential oils (EOs) has been demonstrated as a promising and efficient strategy to address these constraints, leading to improved stability and effectiveness in food systems (Singh et al., 2021; Nejatian et al., 2022).

Therefore, present study has been designed to explore antifungal potential of nanoparticles of essential oils obtained from different parts of Dill plant, against aflatoxin producing fungi and encapsulation of dry fruits and nuts for management of their contamination.

Details of plant

The origins of *Anethum graveolens* (commonly known as Dill) are thought to be in the Mediterranean area. The herb known as dill held significant cultural importance in ancient Greek society and was widely cultivated and recognized. In Greek households, the practice of burning dill scented oil was observed, while the essential oil derived from the plant was employed in the production of certain types of wine. The plant is a herbaceous perennial with little yellow blooms, belonging to the Umbelliferae family. It typically reaches a height of around 1.5 meters. This plant has been historically and contemporarily recognized for its therapeutic properties. Dill originates from the regions of West Asia and the Mediterranean basin. The plant has a wide distribution over several regions, including but not limited to the Middle East, India, Russia, Iran, and Egypt (Katzer, 2009). Seeds are frequently employed in the food business as a flavoring element

due to their highly aromatic and pungent scent. Leaves, on the other hand, find application in salads and tea. However, seeds are regularly utilized in a variety of food products such as tea, bread items, soups, salads, and canned meals. Dill is planted for both its culinary use as a vegetable and its potential as a source of essential oil. The potential medical uses of this volatile oil include its use as a carminative, antispasmodic, stomachic, diuretic, and stimulant. Moreover, the *A. graveolens* plant is frequently employed for the treatment of gastrointestinal ailments such as intestinal and stomach discomfort, liver disorders, dyspepsia, bladder irritation, and cramps (Naseri-Gharib and Heidari, 2007). There was significant variability observed in the antibacterial efficacy of volatile oils and plant extracts. Numerous research has been conducted to elucidate the chemical makeup of the essential oil derived from dill seeds (Gomaa and Gomaa, 2021). The antioxidant properties of the aqueous extracts of *A. graveolens* were compared with those of α-tocopherol, ascorbic acid, and quercetin in in vitro systems. The inhibitory activity of volatile oil derived from shapat a seed against vulvovaginal candidiasis in immunosuppressed rats was documented in a previous study (Badr et al., 2017).

Dill plant (*Anethum graveolens*) (Image courtesy: Google images)

Taxonomic position (Datiles and Acevedo-Rodriguez, 2022**)**

Domain- Eukaryota

Kingdom- Plantae

Phylum- Spermatophyta

Subphylum- Angiospermae

Class- Dicotyledonae

Order- Apiales

Family- Apiaceae

Genus- Anethum

Species- *Anethum graveolens*

2. Review of literature

Dried fruits and nuts are often regarded as a very valuable source of important nutrients due to their provision of protein, fatty acids, potassium, dietary fibers, and bioactive substances. In addition, it has been shown that these advantages extend to human health, since they have the potential to mitigate the risk of obesity, cardiovascular diseases, and diabetes (Carughi et al., 2015). According to Masood et al. (2015), dried fruits and nuts possess a longer shelf life in comparison to fresh fruits, making them a favorable substitute for prolonged consumption. The economic stability of a nation is predominantly contingent upon its export sector, with many developing nations relying heavily on the exportation of agricultural goods as a fundamental pillar of their economic framework (Alghalibi and Shater, 2004). The significance of dried fruits and nuts in this context is noteworthy, as evidenced by the United States generating an annual export revenue of over $3 billion from these products (Johnson et al., 2009; Hussian et al., 2015).

Regrettably, these very expensive food items are susceptible to contamination and spoilage caused by microbes (Barth et al., 2009). Numerous types of fungi, including "*Alternaria, Aspergillus, Candida, Fusarium, Mucor, Rhizopus, Penicillium"*, and others, have the potential to cause food spoilage. Moreover, their presence and consumption in food can give rise to various health-related problems, such as mycoses. These mycoses can manifest as clinical conditions ranging from mild to severe, and can pose a particularly significant risk to individuals with compromised immune systems (Tournas et al., 2015). In recent times, there has been a growing inclination towards the utilization of plant essential oils as agents for controlling the degradation of food products caused by fungus, bacteria, insects, and rodents. These oils have demonstrated potential as fungicides, bactericides, insecticides, and rodenticides. Nevertheless, the inherent volatility of essential oils and their susceptibility to physico-chemical deterioration caused by abiotic conditions impose constraints on their prolonged utilization. Hence, the utilization of nano-based delivery systems may offer an effective approach for pathogen management, since they may

employ several mechanisms to target and disrupt cellular function, ultimately resulting in cell lysis and eventual mortality. The reduced dimensions of nanoparticles enable their intracellular migration, resulting in the impairment of cellular functionality. Moreover, it has been witnessed that the lipophilic chemical constituents included in essential oils possess the ability to readily traverse the plasma membrane of fungi and insects. This process subsequently leads to various enzymatic modifications and biochemical disruptions within their physiological systems (Lee et al., 2004). The active reporting of the inhibition of fungal membrane ergosterol by several essential oils has been documented by Tian et al. (2012). The use of essential oils as bio preservatives and natural antioxidants in food matrices is a very significant area of study in contemporary times. The encapsulation technology that is now undergoing fast development primarily targets several aspects of the food industry, including food processing, packaging, transportation, shelf-life extension, and the improvement of nutritional component bioavailability. Regrettably, it must be noted that essential oils (EOs) are lipophilic substances that are susceptible to degradation due to the influence of temperature, light, moisture and oxygen. Nano-encapsulation is a recognized and effective approach for surmounting these challenges. The use of this approach enables the safeguarding of essential oils from thermal and photo-degradation processes, hence enhancing their solubility in aquatic settings, concealing their taste, and augmenting their bio-accessibility and bioavailability (Gupta and Variyar, 2016). The concentration of EO is enhanced in the region where microorganisms are mostly found, such as water-rich phases or liquid-solid interfaces, due to the subcellular size and relatively greater surface area per unit volume (Weiss et al., 2009).According to Teodoro et al. (2014), the utilization of microencapsulated essential oils with antimicrobial properties has been implemented in smart packaging systems within the food industry, proving to be an efficient strategy. The utilization of nano-encapsulation techniques for essential oils (EOs) represents a cutting-edge approach in the field of delivery strategies. This approach aims to enhance the antimicrobial effectiveness of EOs by improving their solubility, stability, and controlled

release of aromatic compounds within the food system. Additionally, nano-encapsulation provides protection against environmental factors such as light, oxygen, moisture, and pH, thereby further preserving the efficacy of EOs (Pisoschi et al., 2018; Amiri et al., 2020; Delshadi et al., 2020). Various biopolymeric matrices, such as chitosan, dextran, starch, cellulose, and alginate, have been employed as carrier agents for encapsulating essential oils (EO). This choice is attributed to their desirable characteristics, including biodegradability, biocompatibility, and non-toxicity (Hosseini & Meimandipour, 2018). Hence, it can be argued that encapsulated essential oils (EOs) have the potential to serve as superior alternatives to synthetic preservatives in the domains of agriculture and the food industries, due to their non-toxic nature and environmentally beneficial characteristics. The utilization of active food packaging and polymer-based coatings using essential oils (EOs) has garnered recognition as a potential method for delivering food products. This strategy generates a protective barrier that effectively safeguards food against microbial infestation and toxin generation, hence contributing to the extension of food shelf life. The utilization of essential oils (EOs) in food products as environmentally friendly preservatives has been extensively studied. These studies have focused on elucidating the antibacterial mechanism of action of EOs and exploring various contemporary delivery methods that enable regulated release of volatile EOs, ensuring sustained efficacy in the food system. This research highlights the potential of EOs as intelligent and sustainable preservatives for the food industry. In the year 2020, a group of researchers developed a droplet emulsion of essential oil (EO) combined with gold nanoparticles (NP). Additionally, they employed nanoparticles for the encapsulation of peppermint essential oils and cinnamaldehyde (Liakos et al., 2018). In a similar way, the essential oil (EO) of *Lippia sidoides*, which contains thymols, has been subjected to nano-encapsulation using chitosan-gum nanoparticles. These nano-encapsulated thymols find use in the chemical, pharmaceutical, and food sectors (Paula et al., 2010). Researchers have successfully synthesized several derivatives of *Santolina insularis* essential oil, including γ-terpinene, carvacrol, p-cymene, and thymol, as well as combinations of these compounds with

phosphatidylcholine liposome NPs. These preparations have demonstrated efficacy in inhibiting microbial growth, as reported in a study (Valenti et al., 2001). In their respective studies, Aguitar et al. (2015) and Pesavento et al. (2015) conducted research on the antibacterial properties of essential oils derived from clove basil, oregano, rosemary, and thyme. Their investigations focused on evaluating the efficacy of these essential oils against various bacterial strains, including "*Escherichia coli, Pseudomonas aeruginosa, Listeria spp* and *Staphylococcus aureus"*. The antibacterial action of essential oils (EOs) is diminished due to their limited water solubility and oxidizability. According to a research, nano-sized particles are prepared by encapsulating essential oils (EOs) with an appropriate wall material. This encapsulation process serves to enhance the water solubility of the EOs, as well as increase the stability of their biological activity. Consequently, the antibacterial characteristics of the EOs are enhanced (Hamed et al., 2012). The study conducted by Salvia et al. (Salvia-Trujillo et al., 2015) shown that the utilization of nano-emulsification to emulsify citronella oil and clove essential oils resulted in a more potent antibacterial activity against *Escherichia coli.* The impact of surfactant and oil phase composition on the antibacterial efficacy of eugenol cyanophenol nanoemulsion was investigated by Li et al. (2015). The results of the study indicate that the antibacterial activity was enhanced following the process of nano-emulsification. During the nano-emulsification process of eugenol, the inclusion of the ionic surfactant, Tween-80, results in a nano-emulsion with enhanced antibacterial activity compared to the non-ionic surfactant, dodecyl sulfate, when incorporated during the ripening stage. Furthermore, it has been shown that a decrease in the quantity of soybean oil included into clove oil results in an enhanced bacteriostatic effect against *Escherichia coli*. The study conducted by Esmaeili et al. (Esmaeili and Asgari, 2015) involved the utilization of chitosan as a means to encapsulate sesame oil into granules of nano-sized dimensions. However, the encapsulated EOs demonstrated a more pronounced antibacterial efficacy against various strains including "*Cactus rod, Escherichia coli, Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus,* and *S. epidermidis"* when compared to the non-encapsulated EOs. A similar scenario was noted

during the examination of the antibacterial efficacy of d-limonene and oregano essential oils (EOs) subsequent to the process of emulsification (Zhang et al., 2014).

In contrast to non-encapsulated essential oils (EOs), encapsulation has been shown to enhance the water solubility of EOs. Additionally, when the droplet size of EOs is reduced to the nanometer scale, it effectively reduces the mass transfer resistance within the nano-scale transport system. Consequently, this facilitates the passive adsorption of EO droplets by somatic cells, thereby strengthening the antibacterial activity of EOs (Donsi et al., 2010). In their study, Acevedo et al. (Acevedo-Fani et al., 2015) observed that the bacteriostatic properties of E. coli were significantly enhanced when emulsified thyme essential oils were subjected to nano-emulsification. In their study, Moghimi et al. (2016) employed the technique of sonication to create nanocapsules containing essential oil (EO) derived from thymus daenensis. Subsequently, a comparison was made between the antibacterial efficacy of nanocapsules and non-encapsulated essential oil (EO) in relation to food spoilage bacteria. The findings of the study indicated that the nanoemulsions of essential oils (EOs) exhibited a minimum inhibitory concentration of 0.4 mg/mL. Furthermore, the antibacterial activity of these nanoemulsions was seen to be tenfold higher compared to the non-encapsulated EOs. Previous research has indicated that the inhibitory impact of limonene and cinnamic acid nanoemulsions on spoilage yeast and Escherichia coli is not significantly influenced by variations in their sizes. This finding is strongly correlated with the composition of the emulsion system (Donsi et al., 2012). In this study, the authors employed the nanoprecipitation technique to produce nano-encapsules that contained the essential oils derived from *Thymus capitatus* and *Origanum vulgare*. The size, polydispersity index, zeta potential, effectiveness of encapsulation, and loading capacity of the colloidal suspensions were assessed. In the study conducted by Kapustová et al. (2021), the researchers evaluated the efficacy of nanosuspensions containing essential oils against a diverse range of fungal strains, encompassing fourteen species from the Ascomycota and Basidiomycota

groups. The antifungal effects of oregano essential oil (EO) nanoemulsions were investigated by Bedoya-Serna et al. (2018). The study found that these nanoemulsions exhibited efficacy against "*Cladosporium sp., Fusarium sp., and Penicillium sp.* The researchers discovered that in the case of *Fusarium sp.,* as compared to *Penicillium sp.",* the antifungal impact of oregano essential oil was improved by encapsulation.

The utilization of β-Cyclodextrin was employed for the purpose of encapsulating essential oils possessing antifungal properties via the creation of an inclusion complex. The efficacy of β-cyclodextrin capsules containing Mexican oregano and clove essential oils (EO) against *Fusarium oxysporum* was demonstrated (Estrada-Cano et al., 2017). The antifungal activity of an inclusion complex of β-cyclodextrin and *Litsea cubeba* essential oil was demonstrated to be efficient against three prominent fungus found in citrus, namely *Penicillium digitatum, Penicillium italicum,* and *Geotrichum citri-aurantii* (Wang et al., 2020).

Weisany and colleagues reported on the antifungal properties exhibited by inorganic nanoparticles that were loaded with essential oils. The authors demonstrated that the fungicidal action against the plant pathogen *Colletotrichum nymphaeae* was increased by encapsulating *Thymus daenensis* and *Anethum graveolens* in silver nanoparticles (Weisany et al., 2019a). In a previous study, the authors demonstrated that copper nanoparticles infused with essential oils derived from thyme and dill exhibited the capability to effectively hinder the growth of mycelium and significantly suppress the germination of conidia belonging to *Colletotrichum nymphaeae* (Weisany et al., 2019b).

In a study conducted by Hofbauerová et al. (2023), the efficacy of oregano and thyme essential oils encapsulated in poly("-caprolactone) nanocapsules (Or-NCs, Th-NCs) was investigated in terms of their protective and disinfectant properties against four environmental microorganisms, namely *Pseudomonas vancouverensis, Flavobacterium sp., Pleurotus eryngii, Purpureocillium lilacinum* etc.

3. Statement of the Research Problem

The use of submicron size materials (1-1000 nm) in a variety of geometric shapes to entrap EOs in lipidic or polymeric materials to preserve and stabilize them and control their release in the product is what is known as nanotechnology in the food business. The preservation of fruits and vegetables, in particular, has led to the utilization of essential oils that have been nano-encapsulated for use in the food business. Some of these items have antioxidant and antibacterial properties that might be useful when applied to meat and meat products. Their enhanced stability, organoleptic affects, flavor, and function are all guaranteed by nano-encapsulation, which also increases the finished product's shelf life. Therefore, it has been suggested that nano-encapsulation of EO of dill may be used to inhibit fungal infections in dry fruits and nuts.

4. Objectives of The Research work

- To analyse mycoflora of some selected dry fruits and detection of toxicogenic fungal strains from them.
- To isolate essential oils (EOs) from different parts of *Anethum graveolens* L. (Dill plant) and their screening for antioxidant and antimicrobial activity against selected fungal strains.
- To standardize selected EOs by phytochemical and GC-MS analysis.
- To synthesize, nanoparticles and their efficacy assessment as antifungal agent and nano-encapsulation with selected EOs to enhance shelf life of selected dry fruits.

5. Methodology

- Mycoflora analysis of collected dry fruits will be performed by serial dilution and direct plating methods. The obtained fungi will be identified by standard method (Gillman (1998).
- Toxin-producing potential of fungal isolates from dry fruits will be assessed in SMKY medium (Kumar et al., 2010).
- Extraction of EOs from different parts of Dill plant and their screening for the antifungal activity against highly toxic fungal isolates will be done (Chen et al., 2013).

- Phytochemical analysis of EOs will be done and key bioactive compounds will be characterized in most effective EOs by using GC-MS technique (Yang et al., 2021).
- Evaluation of fungitoxicity of test EOs against highly toxic strains will be done in petri plate and their fungitoxic spectrum against common food spoiling fungi will be calculated in terms of MIC and MFC (Dwivedy et al., 2017).
- Antifungal mode of action will be studied using SEM to elucidate the mechanism of antagonism of EOs (Tran et al., 2020).
- Green-Nanoparticles (GNPs) of iron (Fe) and zinc (Zn) will be made using EOs of Dill plant and characterized by standard methods such as XRD, SEM, FTIR and UV-Vis (Rajendran and Sengodan, 2017).
- Antifungal activity of these GNPs will be analysed in terms of MIC and nano-encapsulation of dry fruits will be performed (Diao et al., 2013; Dobrucka and Dugaszewska, 2016).
- Statistical analysis will be performed by ANOVA.

6. Scope of the Study

Valuable foods like nuts and dry fruits are susceptible to contamination and spoilage by microbes (Barth et al., 2009). *Alternaria*, *Aspergillus, Candida, Fusarium, Mucor*, *Rhizopus, Penicillium*, etc. are just some of the many fungi that can grow in food and cause a wide variety of health problems, from mild to potentially fatal clinical conditions known as mycoses. Sadly, EOs are lipophilic chemicals, thus they quickly deteriorate when exposed to oxygen, light, moisture, and heat. Nano-encapsulation technology, which has recently been used to produce EO formulations, has garnered a lot of interest in the food industry. There are a number of benefits that these formulations can offer over traditional food preservation techniques. They ensure the enhanced stability, organoleptic affects, flavor, and function of the finished product by controlling the release of essential oils and protecting them from heat and photo-degradation.

7. Significance of the Study

The rising incidence of fungal contamination of dry fruits and their accompanying toxins during processing and storage has created a risk for stakeholders and consumers alike, underscoring the importance of the current study. The use of EOs has been shown to be effective in this regard, although they do have the potential downside of altering the organoleptic qualities of food items due to the interplay of their different components. The purpose of this research is to identify the essential bioactive components in Dill EOs and investigate their potential biological activity as an antifungal agent against the fungal contaminants isolated from selected dried fruits. Also, the mechanism of action of EOs will be studied using SEM which will further contribute to design more effective combinations to combat the risk associated with fungal contamination in dry fruits. The nano-encapsulation of dry fruits with selected EOs will ensure improvement in shelf life and maintain their quality. It will also be helpful in replacing already present chemical preservatives which are currently considered a significant downside by consumers due to the potentially severe health risks of carcinogenicity and diseases of the gastrointestinal tract. Also, as a future perspective, it is foreseen that the optimization of factors affecting the encapsulation process would lead to a cost-effective and well encapsulated ingredient with a higher efficiency than its free form to extend the food shelf-life.

8. Limitations

As it will be lab study, the performance of the EOs will depend on the environmental factors, age of plant material selected and season. Also, the toxicity and safety assessment will be needed prior to their industrial application.

9. Month Wise Plan of Work

1-12 months	To analyse mycoflora of some selected dry fruits and detection of toxicogenic fungal strains from them.
10-20 months	To isolate essential oils (EOs) from different parts of *Anethum graveolens* L. (Dill plant) and their screening for antioxidant and antimicrobial activity against selected fungal strains.
15-20 months	To standardize selected EOs by phytochemical and GC-MS analysis.
21-30 months	To synthesize, nanoparticles and their efficacy assessment as antifungal agent and nano-encapsulation with selected EOs to enhance shelf life of selected dry fruits.
24-36 months	Statistical analysis, paper and thesis writing

Total Time (expected) = 36 Months

10. WORK PLAN (GRAPHICAL REPRESENTATION)

Objectives	Months					
	0-6	7-12	13-18	19-24	25-30	31-36
To analyse mycoflora of some selected dry fruits and detection of toxicogenic fungal strains from them.						
To isolate essential oils (EOs) from different parts of *Anethum graveolens* L. (Dill plant) and their screening for antioxidant and antimicrobial activity against selected fungal strains						
To standardize selected EOs by phytochemical and GC-MS analysis.						
To synthesize, nanoparticles and their efficacy assessment as antifungal agent and nano-encapsulation with selected EOs to enhance shelf life of selected dry fruits.						
Thesis writing and paper publication						

11. References

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